# Weeds

VOLUME II
JULY 1953
NUMBER 3

*Journal of the Association of Regional Weed Control Conferences*

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Weeds is a quarterly journal published by the Association of Regional Weed Control Conferences. Editorial officers are temporarily located in Room 116, Plant Industry Station, Beltsville, Md. Printing is by the W. F. Humphrey Press, Inc., Geneva, New York. Subscription price is $4.00 yearly or four issues; single copies $1.25. Address all communications regarding subscriptions to Dr. R. L. Lovvorn, Division of Weed Investigations, Bureau of Plant Industry, U.S.D.A., Beltsville, Maryland. For information on advertising contact Dr. H. L. Yowell, Esso Laboratories, P. O. Box 51, Linden, New Jersey. Manuscripts and inquiries on material for publication should be addressed to the Editorial offices.

Entered as second-class matter at the post office at Ithaca, New York, and Geneva, New York.

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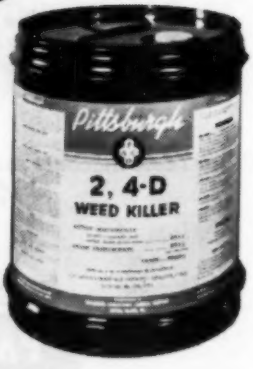


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Weeds

JOURNAL OF THE ASSOCIATION OF REGIONAL WEED CONTROL CONFERENCES.

VOLUME II JULY 1953 NUMBER 3

# Action of Isopropyl N-Phenyl Carbamate Under Various Conditions[1]

A. S. NEWMAN and H. T. DERIGO[2]

ISOPROPYL N-phenyl carbamate (IPC) has been used to a limited extent during the past few years as a selective herbicide in the control of weedy grasses and certain broadleaf species. Some investigators have found IPC to be highly effective; others have found IPC to be ineffective. The present investigation and those of Freed (6) indicate that environmental conditions are extremely important in determining the extent of plant response. Shaw and Swanson (8) found that the herbicidal effectiveness of IPC was highly variable under greenhouse conditions.

It is to be expected that IPC will be most effective under conditions favorable for its persistence. IPC may disappear from soil as the result of microbial action and volatilization. Leaching beyond the root zone and absorption by soil constituents also may reduce the effectiveness of IPC. These last two factors probably are less important in most instances than volatilization or microbial decomposition.

Newman, DeRose, and DeRigo (7) found that IPC persisted more than 9 weeks in autoclaved soils and less than 2 weeks in nonautoclaved soils, which established microbial action as a factor in the disappearance of this compound. Under field conditions the persistence of IPC has been reported to be from five weeks (10) to 30 to 60 days (11). Of course, the concentration of IPC may be greatly reduced in appreciably less time. DeRose (4) found isopropyl N-(3-chlorophenyl) carbamate (3-Cl IPC) to persist more than 48 but less than 54 days and IPC to persist more than 16 but less than 24 days in a greenhouse experiment. Stevens and Carlson (9) in a field experiment found that 2 pounds per acre of IPC persisted more than 2 and less than 4 weeks and 3-Cl IPC persisted more than 6 and less than 8 weeks. Soil temperature has been shown to be a factor in

[1]Contribution from the Biological Laboratories, Chemical Corps, Camp Detrick, Frederick, Md. This paper includes data from a thesis submitted by the junior author in partial fulfillment of the requirements for the degree of Master of Science, University of Rhode Island. The assistance of Dr. T. E. Odland, who supervised the work at Rhode Island, and the staff of the Statistical Branch, Camp Detrick, who made the statistical analyses, is gratefully acknowledged. Presented before Section VI, American Society of Agronomy, Cincinnati, Ohio, November 18, 1952.

[2]Soil Scientist, and Research Agronomist, respectively.

the disappearance of IPC (7) and 3-Cl IPC (9), both compounds disappearing more rapidly at higher temperatures. Stevens and Carlson (9) also found that the persistence of 3-Cl IPC was influenced by soil pH and composition.

Anderson, Linder, and Mitchell (2) have shown that IPC and 3-Cl IPC are sublimed relatively rapidly from glass slides or from tinfoil at temperatures above 80°F. In one experiment 92 percent of the 3-Cl IPC and 80 percent of the IPC coatings on glass slides were lost by sublimation in 24 hours at 85°F. Shaw and Swanson (8) observed that IPC, 3-Cl IPC, and other carbamates applied as pre-emergence sprays under greenhouse and field conditions were volatile and that the vapors possessed herbicidal activity.

The purpose of these investigations was to ascertain the effectiveness of soil applications of IPC as a herbicide under diverse environmental conditions at many stages of plant development and secondarily to compare various formulations of IPC, 3-Cl IPC, and N-(4-chlorophenyl)-N′, N′-dimethyl urea (CMU). Cereals were employed as simulants for weedy grasses in order to obtain precise data on the amount of injury.

## MATERIALS AND METHODS

At Frederick, Maryland the experimental crops were grown on Duffield silt loam, Duffield loam, and Athol gravelly loam. The pH of the experimental areas ranged from 5.9 to 7.1. At Kingston, Rhode Island the crops were grown on Bridgehampton very fine sandy loam. Normal cultural and fertilization practices were followed at both locations. In order to prevent contamination of adjacent plots, level areas were selected for the experiments and the plots were diked.

The plots were 6 rows by 18 feet long, of which the 4 center rows by 16.5 feet were harvested and threshed. The experiments were either randomized complete block or split plot design with 4 replicates, except for the experiment reported on Table 2 which had 3 replicates. Treatments were applied at two rates, except for the experiment reported in Table 6 in which a single rate was used. In Tables 3, 4, and 5 data are given only for the rate at which yield reductions most nearly approached 50 percent. The analyses of variance of the data in Tables 1, 2, and 3 were made on the actual yields, and in Tables 4, 5, and 6 were made on the logarithmic transformation of the yields as percent of control.

The IPC and 3-Cl IPC were obtained from the Columbia-Southern Chemical Corporation. The CMU was obtained from E. I. duPont de Nemours and Co. The sprays of IPC and 3-Cl IPC were prepared by dissolving the carbamates in 6 times their weight of Tween 85 and then pouring these mixtures into warm water. The spray of CMU was prepared by dispersing the 80 percent active ingredient wettable powder in water. The sprays were applied at a volume of 150 ml. per square yard with a 3-gallon knapsack type sprayer. Insofar as practical, only the middles were sprayed.

The 12.5 percent IPC extruded particles were prepared by mixing together 12.5 g. IPC, 72.5 g. fuller's earth, and 5 g. cornstarch to

which was added 10 g. of glucose in solution to form a paste. The 75 percent IPC extruded particles were prepared by mixing 75 g. IPC and 15 g. fuller's earth (or 10 g. fuller's earth plus 5 g. cornstarch), to which was added 10 g. of glucose in solution to form a paste. These pastes were forced through screens having openings $^1/_{16}$ inch in diameter. The extruded particles were dried at 50°C and broken into segments averaging 2.6 to 5.8 mg.

Tablets of IPC contained 75 g. of IPC, 10 g. fuller's earth, 14 g. cornstarch, and 1 g. Tergitol 7. Tablets of 3-Cl IPC contained 75 g. 3-Cl IPC, 24 g. fuller's earth, and 1 g. Tergitol 7. Tablets of CMU contained 75 g. CMU (100 percent active ingredient), 19 g. fuller's earth, 5 g. cornstarch, and 1 g. Tergitol 7. The ingredients were thoroughly blended together and then the tablets were punched out with a Stoke's Eureka model tablet machine. The tablets were $^1/_8$ inch in diameter by approximately $^1/_{16}$ inch in length and the average weight varied from 9 to 11 mg. For each herbicide the composition shown above was the best formulation on basis of ease of preparation of the tablets and of rapidity of dispersion in water.

For both tablets and extruded particles the active ingredient was 40 mesh or smaller. Tablets and extruded particles were applied to the plots by hand broadcasting. In some cases nonherbicidal extruded particles were used as a diluent to aid in obtaining uniform distribution.

## RESULTS AND DISCUSSION

Several investigators have shown that the susceptibility of cereals to injury from soil treatment with IPC decreases with increasing plant age (1, 5, 6). Allard, et al (1) found greater injury of oats at the early seedling stage than at planting time. In the experiments shown in Table I, IPC was equally injurious to oats at emergence and at the 3 to 5 inch stage. At the 8 to 16 inch stage, IPC was highly significantly less effective at Frederick and noneffective at Kingston. In parallel studies soil applications of IPC to cereals in the boot stage or older rarely caused extensive injury under field conditions.

The extent of injury from IPC soil treatments may be greatly modified by soil environmental factors. This is illustrated with Clinton oats in Table 2. It is evident that the greatest amount of injury may occur at other than the early seedling stages. In this experiment the greatest injury occurred at the second to fifth leaf stages. These differences among stages were significant at the 0.1 percent level of probability. Following applications at the first leaf stages there were light rains which slowly dispersed the extruded particles. The dispersion of the extruded particles was visually ascertained after each rain. Conditions were favorable for loss of the IPC and the concentration of IPC apparently was markedly reduced prior to its movement down to the root zone. As was mentioned previously, IPC is not a highly persistent compound. At the later stages heavier rains fell shortly after treatment, presumably leaching the carbamate down to the root zone before much loss could take place.

*Table 1.* Reduction in yield (percent) of threshed grain of Clinton spring oats treated with 2.5 and 5.0 pounds per acre of IPC* at two locations in 1949.

| Stage | Location | | | |
|---|---|---|---|---|
| | Frederick | | Kingston | |
| | 2.5 lb. | 5.0 lb. | 2.5 lb. | 5.0 lb. |
| Emergence | 43 | 59 | 31 | 77 |
| 3–5 in. | 35 | 50 | 38 | 73 |
| 8–16 in. | 23 | 27 | −8 | −7 |

*Applied as extruded particles containing 12.5 percent active ingredient.

*Table 2.* Injury of Clinton spring oats following treatment with 2 and 4 pounds per acre of IPC* at Frederick, Maryland in 1950.

| Date | Stage | Percent yield reduction of threshed grain | |
|---|---|---|---|
| | | 2 lb. | 4 lb. |
| 21 Apr. | Early 1st leaf | 13 | 48 |
| 24 Apr. | Late 1st leaf | 25 | 40 |
| 1 May | Early 2nd leaf | 43 | 72 |
| 8 May | 4th leaf | 69 | 79 |
| 17 May | Early 5th leaf | 42 | 73 |
| 22 May | Late 5th leaf | 62 | 78 |

*Applied as extruded particles containing 75 percent active ingredient.

Results similar to those in Table 2 have been obtained in many field experiments at Frederick, Maryland and Kingston, Rhode Island from 1948 through 1952. In these experiments IPC was applied to the soil both as solid particles and as aqueous sprays. As will be shown later, the relative injury at the various stages most likely would have been different if aqueous sprays or dust had been employed.

Soil temperature is an important factor in the response of fall sown cereals to IPC. This is indicated in Table 3. At both Frederick and Kingston, the fall and midwinter treatments were most injurious to wheat and rye. At both locations differences among stages were highly significant. Similar results were obtained with 5 pounds per acre of IPC. The greater injury probably was related to the persistence of IPC. At the low temperatures prevailing during the winter period decomposition of IPC will be slight because of the low activity of the soil microorganisms. Also, loss of IPC by volatilization will be less at the low temperatures. Consequently, the maximal amount of chemical will be available to induce plant response. Ordinarily, a significant portion of the IPC applied during the early fall or spring is lost prior to its being leached down to the root zone. Consequently, treatments at these times are, in general, not as effective as those applied during the cold weather period.

It is possible, of course, that cereal plants and other grass and broadleaf species are most susceptible to IPC injury during their dormant period. A preliminary experiment indicated that a small amount of IPC was absorbed by winter wheat after a one week's exposure, during a period when the air temperature averaged 41°F.

*Table 3.* Reduction in yield (percent) of threshed grain of winter cereals treated with 2.5 pounds per acre of IPC* at two locations during 1948–1949.

| Stage | Frederick | | Kingston | |
|---|---|---|---|---|
| | Thorne wheat | Abruzzi rye | Thorne wheat | Selected rye |
| Emergence | 37 | 37 | 23 | 39 |
| 2–3 leaf | 59 | 76 | 29 | 46 |
| Midwinter | 73 | 91 | 69 | 59 |
| 4–7 leaf | 15 | 25 | 10 | 14 |
| Preboot | 24 | 0 | −14 | 1 |

*Applied as extruded particles containing 12.5 percent active ingredient.

A somewhat longer exposure was required to obtain maximal response. Generally there is no visible top response of cereals treated during the late fall or winter until growth resumes in the spring. If the winter is mild and there is appreciable vegetative growth, top response may be evident within two months after treatment.

The results given in Table 3 are fairly typical of data for four seasons. In 12 of 16 experiments, the greatest injury was produced by late fall or winter treatments. Occasionally early fall or late March and April applications were as effective as winter applications. It is obvious that further research is needed to elucidate the relative effectiveness of IPC at various stages of plant development.

Starting in 1948, comparisons were made of spray, dust, and pellet formulations of IPC. In a 1948 experiment in which IPC was applied at 2 pounds per acre to Clinton spring oats at the two leaf stage, the yield reductions were 22 percent for an aqueous spray and 59 percent for extruded particles. In another 1948 experiment on Thorne winter wheat a spray formulation was superior to an extruded formulation at two different stages. In experiments during the 1949 season it was found that dust and aqueous spray formulations of IPC were generally equally injurious to three cereal species. An extruded formulation was less effective than sprays or dusts in four of five cases. In these experiments the extruded particles contained 12.5 percent active ingredient and dispersed fairly slowly on exposure to rain.

In later experiments extruded particles which dispersed rapidly in water and which contained 75 percent active ingredient were employed. In Table 4 are given the results for an experiment in which such particles were used. In three of six instances IPC extruded

*Table 4.* Reduction in yield* (percent) of threshed grain of Clinton spring oats treated with 2 pounds per acre of IPC or 3-Cl IPC at two locations in 1951.

| Frederick | | | | | Kingston | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Leaf stage | Date | IPC extd | IPC spray | 3-Cl IPC spray | Leaf stage | Date | IPC extd | IPC spray | 3-Cl IPC spray |
| 1st | 9 Apr | 57 | 81 | 69 | 1st | 1 May | 61 | 27 | 54 |
| 2nd | 27 Apr | 74 | 8 | 41 | 3rd | 15 May | 74 | 11 | 55 |
| 5th | 14 May | 13 | 21 | 43 | 5th | 29 May | 9 | 51 | 77 |

*Stage of development and stage-formulation interaction were highly significant at both locations; formulation was highly significant at Kingston and nonsignificant at Frederick. Analysis based on 2 and 4 pound rates.

particles were observably more effective than IPC spray or 3-Cl IPC spray. In two instances the spray formulation of IPC proved superior to the extruded formulation. The spray formulation of 3-Cl IPC was observably best in two cases.

The superiority of one formulation over another appears to be determined in large measure by climatic conditions, particularly soil moisture and temperature. IPC applied to a moist soil as a spray will begin to be decomposed at once and continue until the soil dries out; whereas, IPC applied in extruded particles to the same soil will be little affected by soil microorganisms. Also more of the spray applied IPC will be lost by volatilization. If a light rain falls shortly after the application of these formulations, the extruded particles will be partially dispersed and only a small amount of the IPC will penetrate to the root zone. A much larger amount of the IPC applied as a spray will penetrate to the root zone since the IPC is already evenly dispersed over the surface of the plot. If a heavy rain falls shortly after treatment, the spray and the extruded formulations will be equally effective, because the growth regulator in both cases will move rapidly to the root zone.

In experiments on Clinton oats and Thorne wheat during the 1951–1952 season, results similar to those in Table 4 were obtained. A tablet formulation of IPC was superior to an aqueous spray formulation in 5 of 7 cases (Table 5); the spray formulation was superior in two cases. Statistically, with IPC the tablet formulation was superior; with CMU the spray formulation was superior on oats and tablets were superior on wheat; with 3-Cl IPC the spray formulation was

*Table 5.* Reduction in yield* (percent) of threshed grain of Clinton oats and Thorne wheat treated with tablet and spray formulations of IPC, 3-Cl IPC, and CMU during 1951–1952 at Frederick, Maryland.

| Stage | IPC | | 3-Cl IPC | | CMU | |
|---|---|---|---|---|---|---|
| | Tablet | Spray | Tablet | Spray | Tablet | Spray |
| | Spring oats—3 pounds herbicide per acre | | | | | |
| 1st leaf, 18 Apr. | 77 | 9 | 36 | 32 | 34 | 52 |
| Early 3rd leaf, 1 May | 43 | 14 | 49 | 24 | 30 | 36 |
| 4th leaf, 9 May | 63 | 43 | 58 | 84 | 22 | 47 |
| 5th leaf, 28 May | −7 | 17 | 12 | 52 | 11 | 20 |
| | Winter wheat—2 pounds herbicide per acre | | | | | |
| 1st leaf, 9 Nov. | 27 | 1 | 2 | 6 | 31 | 42 |
| Late 3rd leaf, 25 Jan. | 26 | 58 | 20 | 20 | 25 | 29 |
| 4th leaf, 3 Apr. | 48 | 30 | 39 | 57 | 32 | 21 |

*Stage and all interactions were highly significant and formulation was nonsignificant for both crops; compound was highly significant for oats and nonsignificant for wheat. Analysis for 3 pound rate only for oats; for 1 and 2 pound rates for wheat.

superior. Similar results were obtained with both rates of treatment. The differences between formulations of 3-Cl IPC and CMU were not as well defined as those for IPC. The tablet formulations of IPC and CMU dispersed readily in water, but the tablet formulation of 3-Cl IPC dispersed somewhat slowly, particularly after storage for a few weeks. This slow dispersion of 3-Cl IPC tablets probably affected its relative effectiveness.

In another experiment on Thorne winter wheat the spray formulation of 3-Cl IPC was superior to the spray formulation of IPC in four of six times of application (Table 6). Similar results were obtained during the 1951–1952 season on both oats and wheat (Table 5). The superiority of the spray formulation of 3-Cl IPC in these experiments probably was due to its greater persistence and also to its greater biological activity.

## GENERAL DISCUSSION

The relative importance of volatilization and microbial action in the disappearance of IPC and 3-Cl IPC from soil is not known. Organisms capable of growing on IPC or 3-Cl IPC have been isolated at this laboratory, but their ability to decompose these compounds has not been demonstrated conclusively. High soil temperatures will increase loss by both volatilization and microbial decomposition. In dry soils no microbial decomposition will occur but loss by sublimation may be important. IPC has been shown to disappear equally rapidly from soils at moisture levels from 20 to 80 percent of the water-holding capacity (7).

To obtain the greatest effect from IPC and other readily decomposed or volatilized herbicides, they should be applied when conditions are unfavorable for loss. In the control of established weeds,

*Table 6.* Reduction in yield* (percent) of threshed grain of Thorne winter wheat treated with 2 pounds per acre of IPC and 3-Cl IPC as aqueous sprays at two locations during 1950–1951.

| Frederick | | | Kingston | | |
|---|---|---|---|---|---|
| Stage | IPC | 3-Cl IPC | Stage | IPC | 3-Cl IPC |
| 2nd leaf, 30 Oct. | 23 | 80 | 3rd leaf, 16 Oct. | 1 | 19 |
| 3rd leaf, 12 Jan. | 95 | 99 | 5th leaf, 9 Feb. | 44 | 83 |
| 4th leaf, 21 Mar. | 54 | 97 | 6–8 in., 9 Apr | 28 | 5 |

*Stage of development and compound were highly significant at both locations; stage—compound interaction was highly significant at Kingston and nonsignificant at Frederick.

IPC should not be applied as a spray to a warm soil unless a rain is expected within a few days. If the weeds to be controlled by a herbicide of this type are perennials, winter annuals, or biennials, it is probable that winter treatment will be most injurious.

Soil type influences the action of any soil applied herbicide and it also influences movement within the profile. Unpublished data from this laboratory have shown that IPC is increasingly injurious with increasing percentage of sand in the soil and decreasingly injurious with increasing percentage of organic matter. Blouch and Fults (3) have shown that soil type influences the selectivity of 3-Cl IPC. Further research on the relative effectiveness of herbicides on various soil types is needed.

Further research is needed on pellet formulations of IPC. It appears that properly formulated pellets of IPC generally will be superior to aqueous sprays under average soil moisture and temperature conditions. Properly formulated pellets of IPC should dis-

perse in water in less than 30 seconds. Although commercially formulated pellets might have compositions quite different from those employed in these investigations, it is essential that they have good dispersion properties. The proper size for pellets of this type has not been fully investigated. In these studies the pellets weighed from 2.6 to 11 mg. These sizes appeared to give an adequate number of particles per unit area.

3-Cl IPC and CMU are more persistent in soil than IPC. Consequently, formulation is not as critical a factor with these two compounds. Properly formulated pellets of 3-Cl IPC and CMU should be as effective as spray formulations under most environmental conditions. As was mentioned earlier, the pellet formulation of 3-Cl IPC used in these experiments dispersed somewhat slowly, which probably decreased its effectiveness.

The application of pellets should pose no particularly great problem. Pellets probably could be applied with a modified grain drill or similar type of equipment. For aerial application only minor modifications of presently employed hoppers should be necessary.

## SUMMARY

Soil applications of IPC were made to spring and fall sown cereals at many stages of development at Frederick, Maryland and Kingston, Rhode Island. Limited studies were made with 3-Cl IPC and CMU.

The greatest injury to spring sown cereals from IPC treatment frequently occurred at other than at emergence or early seedling stages. The greatest injury to fall sown cereals generally was produced by late fall or winter applications of IPC. The amount of injury presumably was determined by the variable persistence of IPC and the relative susceptibility of the cereals at the various stages of development.

Pellet formulations of IPC generally were superior to an aqueous spray formulation. An appreciable amount of the spray applied IPC presumably was lost by volatilization or microbial decomposition prior to its being leached down to the root zone.

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# Some Effects of Isopropyl N-(3-Chlorophenyl) Carbamate and an Alkanolamine Salt of Dinitro Ortho Secondary Butyl Phenol on Germinating Cotton Seeds

C. R. Swanson, W. C. Shaw and J. H. Hughes[1]

Many field studies have indicated the relative tolerance of cotton and other crops to certain chemicals used as pre-emergence weed killers. The possibility does exist, however, that the herbicide may cause injury to the crop when applied prior to emergence of the seedlings. The extent of injury will depend upon a number of factors such as rate of application, depth of seeding, penetration of the chemical into the soil, absorption of the chemical by the young plant and inherent tolerance of the species to a given herbicide. Implicit in each field test is the possibility that, under different conditions of rainfall, temperature and soil type, injury to the seedling crop cannot be ruled out. Some factors can be controlled to provide maximum safety to the germinating crop seeds, but others, such as weather conditions, are largely uncontrollable.

Knowledge regarding the true tolerance of germinating crop seeds to an herbicide proposed for pre-emergence weed control is of the greatest importance. Only if the absolute effect of the chemical on the germinating seed is known will there be a basis for predicting crop tolerance under widely varying environmental conditions. Therefore, it is important to know early in the development of any proposed pre-emergence herbicide what the effect of that chemical will be under conditions of direct contact with the seed. This information can be obtained most easily and accurately by means of *in vitro* studies under controlled laboratory conditions.

The importance of isopropyl N-(3-chlorophenyl)carbamate (CIPC) and dinitro ortho secondary butyl phenol (DNBP) as herbicides, and their suggested use for pre-emergence weed control in cotton, indicates the need for study of the effect of these compounds on germinating cotton seeds. Information regarding the mode of action of these chemicals is of equal importance to their judicious use under pre-emergence conditions.

The purpose of the investigations reported here was to study the effects of two important pre-emergence herbicides on germinating cotton seeds and to determine some aspects of their mode of action.

## Review of Literature

The herbicidal properties of dinitro ortho secondary butyl phenol and isopropyl N-(3-chlorophenyl)carbamate are well known. Blackman, Templeman and Halliday (3) have reviewed the literature on the dinitro phenols and the development of the carbamates as herbicides has been reviewed by Freed (7) and by Shaw and Swanson

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(13). Although differing in chemical structure, the carbamates and the dinitro compounds have in common the property of killing many annual weeds in crops when applied pre-emergence. The toxicity of the dinitro phenols to a wide range of organisms has been emphasized by Blackman et al. (3). It is probable that the dinitro compounds act upon a metabolic process common to many plants and animals. Respiration of treated tissue may be stimulated or inhibited depending upon the concentration used (5,11). Dinitro phenol may inhibit growth in plants with a simultaneous stimulation of respiration (4). The stimulatory response of corn root tip respiration to 2,4-dinitrophenol has been demonstrated by Beevers (2) who found that the respiratory quotient (RQ) was markedly increased. He concluded that the high RQ's produced were a result of induction of aerobic fermentation by the dinitrophenol.

More information is available regarding the herbicidal properties than the physiological effects of the carbamates. However, it has been shown by Ennis (6) that isopropyl N-phenyl carbamate acts as a mitotic poison. Studies by Ivens and Blackman (9) indicate that the inhibition of root growth may be a result both of an effect on the spindle of dividing cells and an effect on some metabolic activity connected with mitosis. Their work with ethyl phenylcarbamate suggests that assessment of toxicity of the carbamates in terms of mitotic aberrations alone may give misleading results. Freed (8) stated that the respiration of seedlings treated with isopropyl N-(3-chlorophenyl)-carbamate is initially inhibited, followed by a stimulation and then a gradual decline.

## MATERIALS AND METHODS

The effects of CIPC and DNBP on germinating cotton seed were studied under controlled laboratory conditions. In the present investigations, germination, root elongation and root-tip respiration were considered to be the more important factors in establishment of the young plant. Root-tip respiration and elongation of the radicle are especially good indicators of response of the seedling to treatment.

Each treatment was replicated four times and each experiment was repeated at least three times to assure reproducibility. Acid delinted cotton seed, variety Acala 28, was used uniformly in these experiments. Three percent sodium hypochlorite was employed as a surface sterilant. The seeds were soaked in the hypochlorite solution for one minute and the excess chemical allowed to drain from the seeds before germination experiments were begun.

The chemicals used in these experiments were a concentrate of isopropyl N-(3-chlorophenyl)-carbamate (CIPC) in xylene, emulsifiable in water, and an alkanolamine salt of dinitro ortho secondary butyl phenol (DNBP) formulation containing three pounds of the dinitro ortho secondary butyl phenol per gallon. All rates and concentrations were expressed in terms of the active isopropyl N-(3-chlorophenyl)-carbamate and the dinitro ortho secondary butyl phenol.

*Direct contact treatments*

In the experiments designed to determine the effect of direct contact of the chemicals on response of the seedlings, the cotton seeds were germinated in Petri dishes 15 cm. in diameter. Two filter papers in the bottom of each dish served as the germination stratum. Ten ml. of the herbicide solution of appropriate concentration or ten ml. of distilled water were pipetted onto these filter papers. After placing the surface sterilized seeds in each dish, they were covered with an untreated filter paper. The dish was covered and placed in an incubator. An additional 5 ml. of distilled water was added to each treatment on the second day after initiation of germination to maintain growth. The chemicals were applied to the filter papers in the Petri dishes at rates equalling 0 to 8 pounds phenol equivalent per acre for DNBP and 0 to 16 pounds per acre for CIPC calculated on a surface area basis.

*Vapor treatments*

In the experiments on the effects of the vapors of the two herbicides on germination and root elongation of cotton seeds, the procedure was similar except that the chemicals were applied only to a filter paper fitted into the top of the Petri dish cover. The size of the filter paper was adjusted so that it was held up by the upper rim of the lower half of the Petri dish. There was a one inch separation between the germinating seeds and the treated filter paper.

Germination was carried out in a constant temperature incubator maintained at 30°C during the day and 20°C at night. All measurements were taken 72 hours after the seeds were placed in the incubator. Root length measurements were made to the nearest millimeter. The seeds were considered germinated when the radicle was in excess of 1.5 mm. long.

*Respiration*

Respiration experiments were carried out with the conventional Warburg apparatus using the direct method of Warburg to determine oxygen uptake and carbon dioxide evolution. All measurements were made at a bath temperature of 28.0°C. Figure 1 shows part of the experimental equipment. Root tips from the seedlings grown in contact with CIPC or DNBP were cut to approximately 1 cm. in length, weighed, and placed in the main reaction portion of each flask. The center well contained 0.2 ml. of 20% KOH or 0.2 ml. of distilled water, along with an accordion-folded filter paper to increase the absorbing surface. Filter paper disks were cut to fit the bottom of the flasks and 0.5 ml. of distilled water were added to each flask. In this way the flask atmosphere was kept sufficiently moist to maintain turgor of the root tips. This method (12) was used instead of suspending the root tips in water because immersion in water greatly impedes normal gas exchange.

In later experiments designed to determine the initial effect of the chemicals on the respiration of untreated root tips, the herbicide solutions were added to the tissue from a side arm during the ex-

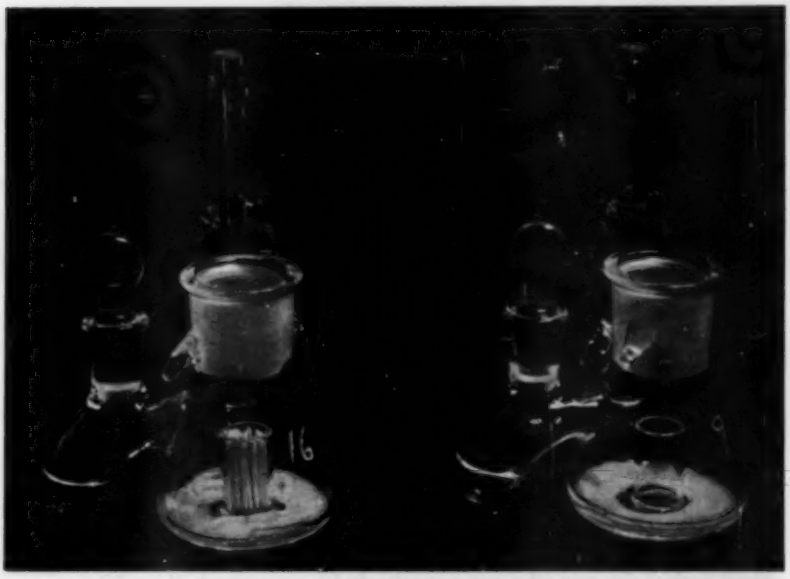

FIG. 1. Warburg flasks used in respiration studies. Root tips were placed in the main reaction portion of the flask. The center well contained alkali in oxygen absorption determinations and in those studies where the initial effects of the inhibitor were determined, the chemicals were tipped in from the side arm.

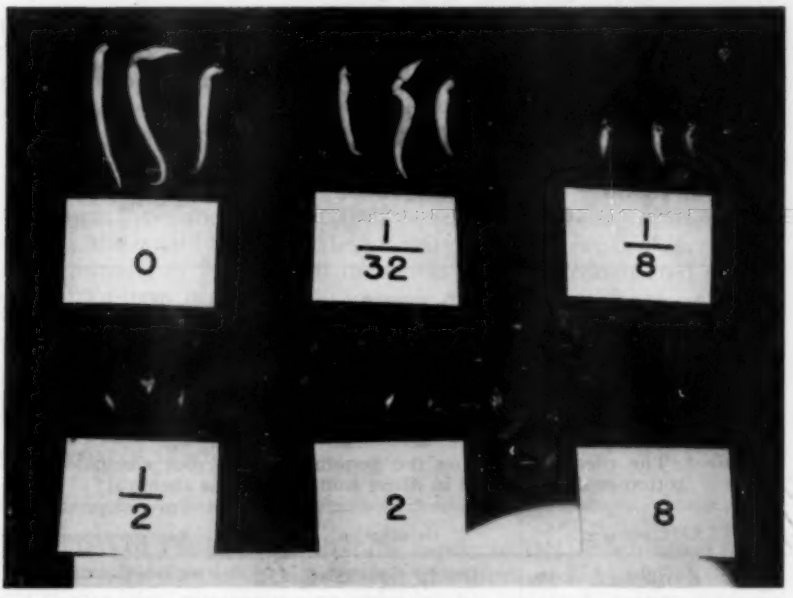


FIG. 2. The effect of DNBP on the root elongation of cotton seedlings grown in contact with the chemical. Rates of application were 0, 1/32, 1/8, 1/2, 2 and 8 pounds per acre.

periment after the normal rate of respiration was established. Concentrations of chemical were expressed as the final concentration after dilution in the Warburg flasks.

## RESULTS AND DISCUSSION

It is important to know the weed-killing properties of a pre-emergence herbicide but it is equally important to know how the germinating crop is affected. The data presented here indicate the growth and respiratory response of cotton seedlings to two important pre-emergence herbicides in the laboratory. A study was made of the effect of direct contact application of CIPC and DNBP on germination, root elongation and root tip respiration. Experiments were also conducted in which the germinating seeds were exposed to the vapors of each of these chemicals.

### *Germination and Root Elongation*

#### *Direct contact treatments*

The data in Table 1 are the means of four replications of an experiment repeated three times. These results are typical of the response of the germinating cotton seeds in direct contact with DNBP. The clearly toxic effect of DNBP is shown in Figure 2. The mean lethal dosage (the rate at which 50 per cent kill or 50 per cent depression of growth is obtained) was approximately 1/8 pound per acre. Rates in excess of 1/2 to one pound per acre in direct contact with the seed caused almost complete inhibition of germination.

*Table 1.* The effect of DNBP on the germination and root elongation of cotton seed germinated in direct contact with the chemical.

| DNBP per acre | Germination | Root elongation |
|---|---|---|
| (lbs) | (percent of untreated) | (percent of untreated) |
| 0 | 100 | 100 |
| 1/32 | 91 | 70 |
| 1/8 | 52 | 22 |
| 1/2 | 18 | 14 |
| 2 | 0 | 0 |
| 8 | 0 | 0 |

These data indicate that loss in germination and reduction in growth vigor could be expected when the seeds are in continued contact with rates in excess of 1/32 pound per acre.

Germinating cotton seeds were markedly more tolerant to CIPC than to DNBP as indicated in Table 2 and Figure 3. At rates up to

*Table 2.* The effect of CIPC on the germination and root elongation of cotton seed germinated in direct contact with the chemical.

| CIPC per acre | Germination | Root elongation |
|---|---|---|
| (lbs) | (percent of untreated) | (percent of untreated) |
| 0 | 100 | 100 |
| 1.6 | 100 | 56 |
| 8 | 103 | 60 |
| 16 | 101 | 52 |

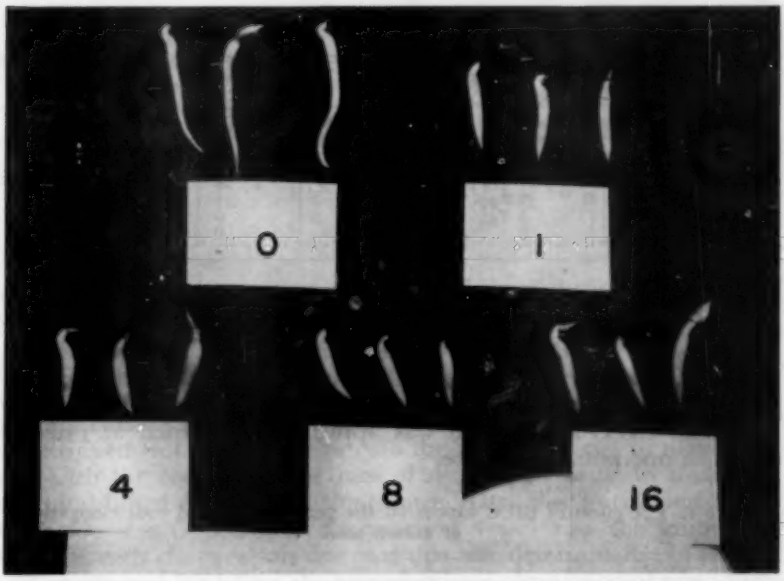


FIG. 3. The effect of CIPC on the root elongation of cotton seedlings grown in contact with the chemical. Rates of application were 0, 1, 4, 8 and 16 pounds per acre.

16 pounds of CIPC per acre there was no inhibition of germination. CIPC produced a marked depression of root elongation but the degree of inhibition was independent of rate or concentration. A mean lethal dosage cannot be given for CIPC because there was no decrease in germination, and inhibition of root elongation was not a function of concentration within the range studied.

The young treated seedlings shown in Figure 3 indicate the type of abnormality produced by CIPC. In addition to being shorter than normal, the entire length of the primary root was swollen except for the terminal 1 or 2 mm., and there was usually a constricted region at the root-stem juncture. Microscopic examination of a number of transverse sections of CIPC treated roots showed no anatomical variations from normal, other than cell size. The cortical parenchyma cells were 2 to 3 times the diameter of comparable cells from untreated roots. The number of rows of cells of the cortex was identical in each case. This suggests that the process of cell elongation may be selectively affected.

*Vapor treatments*

The data of Table 3 show that the vapors of DNBP inhibited both the germination and root elongation of cotton seed. As would be expected, the level of activity was considerably below that of the chemical in direct contact with the seed. However, the vapor activity was sufficient to lower the overall seedling vigor.

*Table 3.* The comparative effects of DNBP as a vapor and as a contact treatment on the germination and root elongation of cotton seed.

| DNBP per acre | Germination | | Root elongation | |
|---|---|---|---|---|
| | Contact | Vapors | Contact | Vapors |
| (lbs) | (percent of untreated) | | (percent of untreated) | |
| 0 | 100 | 100 | 100 | 100 |
| 1/32 | 91 | 97 | 70 | 94 |
| 1/4 | 37 | 97 | 15 | 85 |
| 2 | 0 | 93 | 0 | 69 |
| 8 | 0 | 89 | 0 | 68 |

The vapor activity of CIPC is shown in Table 4. The nature of injury by the vapors of CIPC was of the same type as that of direct contact with the chemical. There was no effect on germination. However, root elongation was inhibited by a constant amount regardless of rate of application. Inhibition of root elongation by vapors of CIPC and DNBP was approximately equal at rates in excess of one pound per acre.

*Table 4.* The effect of CIPC vapors on the germination and root elongation of cotton seed.

| CPIC per acre | Germination | Root elongation |
|---|---|---|
| (lbs) | (percent of untreated) | (percent of untreated) |
| 0 | 100 | 100 |
| 1 | 110 | 74 |
| 4 | 100 | 69 |
| 16 | 99 | 72 |

### *Respiration*

Studies of root-tip respiration were conducted to determine the effect of continued contact with sublethal dosages of CIPC and DNBP on the metabolic vigor of cotton seedlings. These investigations were necessarily limited to determinations of gas exchange of root-tips grown in contact with rates of chemical low enough to avoid complete or severe inhibition of germination and root elongation. Rates higher than $^1/_{32}$ pound per acre of DNBP produced severely malformed and shortened roots which were unsatisfactory for study. CIPC treated roots were straight and of uniform size over the entire range of concentrations. Therefore, since the two chemicals varied so widely in their activity and mode of action, equivalent rates could not be studied. Insofar as possible, rates producing approximately equivalent injury were investigated.

The data of Table 5 indicate the effect of CIPC and DNBP on the respiration of cotton root tips after 72 hours of direct contact with the chemical. At $^1/_{32}$ pound per acre of the dinitro there was a slight but consistent stimulation of both oxygen uptake and carbon dioxide evolution, with no important change in the respiratory quotient. CIPC produced a definite depression of respiration at the two rates investigated. At 1.6 pounds per acre there was a 20 per cent depression of oxygen consumption and at 8 pounds, oxygen uptake

*Table 5.* Respiration of cotton root tips grown in contact with CIPC and DNBP.

| Treatment | | $O_2$ absorbed | $CO_2$ evolved |
|---|---|---|---|
| Chemical | Rate per acre | | |
| | (lbs) | (percent of untreated) | |
| Untreated | 0 | 100 | 100 |
| DNPB | 1/32 | 112 | 108 |
| CIPC | 1.6 | 80 | 88 |
| CIPC | 8.0 | 62 | 56 |

was inhibited by 40 per cent. Carbon dioxide evolution followed the same trend.

In order to obtain the initial effect of these compounds on root-tip respiration, untreated root tips were placed in the reaction portion of Warburg flasks. Thirty minutes after the beginning of each experiment, 0.5 ml. of CIPC or DNBP of the desired concentration were tipped from the side arm into the reaction vessel to make contact with the root tips. The rate of respiration prior to treatment was established for the first 30 minutes. After this initial period the herbicide was tipped into the reaction vessel and the immediate effect of each chemical on the root tips was determined.

The effect of DNBP on the rate of oxygen absorption of previously untreated cotton root tips is presented in Figure 4. Each point on the graph is the mean of an experiment replicated twice and repeated three times. In addition to the untreated controls, three concentrations of DNBP were used – 0.72, 72, 7,200 ppm. The highest concentration used was approximately equal in concentration to the 8 pound per acre treatments mentioned earlier in this report. There was a gradual slight decrease in the rate of respiration of the untreated tissue with time, as indicated in Figure 4. The lowest concentration of DNBP, 0.72 ppm., had no inhibitory effect and produced a slight stimulation as compared to the untreated control. At 72 ppm. there was an immediate depression followed by a gradual decrease in rate of oxygen uptake for the duration of the experiment. Within two hours, the rate of oxygen uptake had been cut in half and after three hours, oxygen uptake was less than one third that of the controls. Response of the root tips to 7,200 ppm. DNBP was immediate and severe. Within half an hour after treatment the rate of oxygen absorption was one fifth that of the untreated and within an hour the rate had dropped to less than one tenth that of the controls. This low rate was maintained for the remainder of the experimental period. Carbon dioxide evolution followed similar trends but at 7,200 ppm., $CO_2$ was depressed to a greater extent than $O_2$, with a resultant average respiratory quotient (RQ) of 0.75. At 72 ppm. DNBP, $CO_2$ was inhibited less than $O_2$, yielding an RQ of 1.2. The RQ of untreated tissue averaged 0.97.

During the course of experiments to determine the effect of CIPC on oxygen uptake and carbon dioxide evolution it became evident that a reaction was taking place in the flasks in addition to the effect of the chemical on the gas exchange of the root tissue.

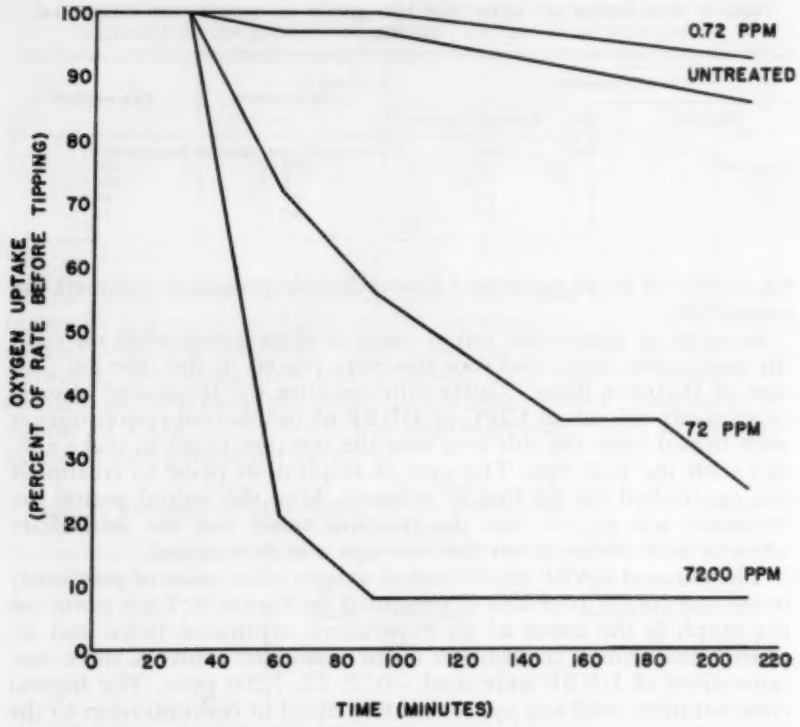


FIG. 4. Respiration of cotton root tips treated with several concentrations of DNBP. The chemical was added to the root tips 30 minutes after each experiment was initiated.

When dilute CIPC was added to the root tips respiring in water a complete reversal of the manometric curve was noted. This was followed by a return to a rate near that of the original within 20 minutes. The gas evolved was not fixed by alkali, indicating that the excess pressure was not due solely to carbon dioxide. James and Garton (10) found a very similar reaction when using sodium diethyldithiocarbamate, and attributed the evolution of gas to production of carbon oxysulfide upon breakdown of the carbamate. In the case of CIPC it is possible that the sudden pressure was due to vaporization of the solvent or perhaps to alteration of gas solubility in the unbuffered solutions. An identical reaction occurred when no root tips were present. Therefore, it was possible to calculate an approximate rate of oxygen consumption and carbon dioxide evolution after treatment.

Pronounced inhibition of oxygen uptake was noted when CIPC was added to untreated excised cotton root tips. Treatments were 31, 310, and 3,100 ppm. of CIPC added to the root tips after the normal respiratory level was determined. As shown in Figure 5, there was no inhibition of oxygen uptake at 31 ppm. The apparent

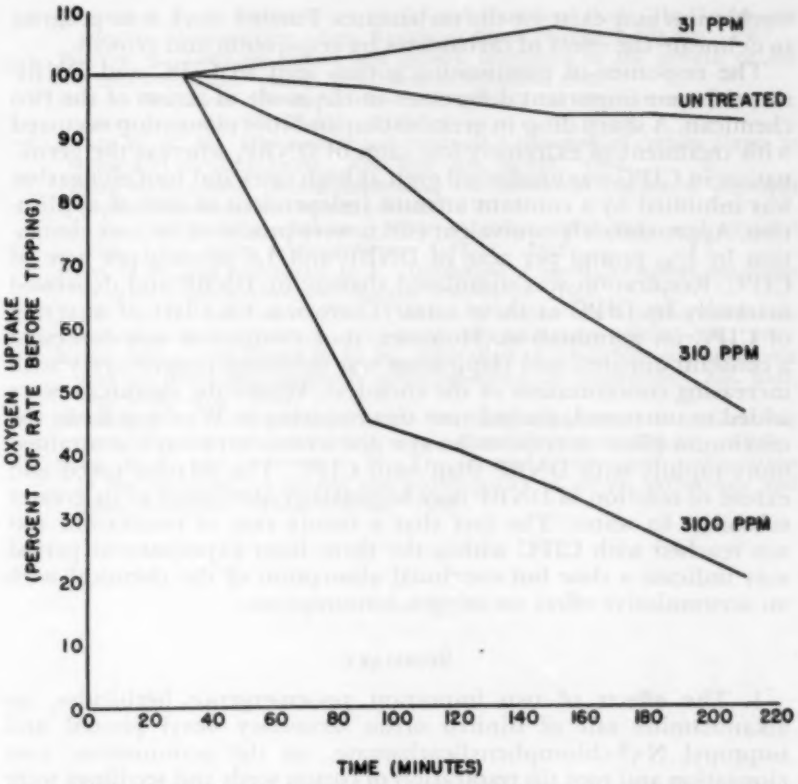


FIG. 5. Respiration of cotton root tips treated with several concentrations of CIPC. The chemical was added to the root tips 30 minutes after each experiment was initiated.

slight stimulation at this concentration was nullified by the fact that addition of a suspension of the solvent alone produced a slight stimulation of oxygen absorption. At 310 and 3,100 ppm. of CIPC there was a progressive inhibition of oxygen uptake with time after addition of the chemical. The relatively greater inhibition of oxygen uptake than of carbon dioxide evolution is indicated by the respiratory quotients obtained. The mean RQ of untreated tissue was 1.07 and that of root tips treated with 31 ppm. was 1.06. Root tips treated with 310 ppm. had an initial RQ of 1.08 which increased to 1.37 three hours after treatment. At 3,100 ppm., the mean RQ was 1.62.

The high RQ obtained by treatment with CIPC may indicate that aerobic fermentation is induced in treated cotton root tips. Beevers has demonstrated that in corn root tips treated with malonate (2) and 2,4-dinitrophenol (1) there is an induction of aerobic fermentation. The similarity of respiratory response of CIPC treated tissue to that treated with malonate or dinitrophenol suggests that a similar

mechanism may exist for the carbamates. Further work is in progress to delineate the effect of carbamates on respiration and growth.

The responses of germinating cotton seed to CIPC and DNBP showed some important differences in the mode of action of the two chemicals. A sharp drop in germination and root elongation occurred with treatment of extremely low rates of DNBP, whereas the germination in CIPC was unaffected even at high rates and root elongation was inhibited by a constant amount independent of rate of application. Approximately equivalent effects were produced on root elongation by $^1/_{32}$ pound per acre of DNBP and 1.6 pounds per acre of CIPC. Respiration was stimulated slightly by DNBP and depressed markedly by CIPC at these rates. There was no effect of any rate of CIPC on germination. However, root elongation was depressed a constant amount, and respiration was inhibited progressively with increasing concentration of the chemical. Where the chemicals were added to untreated, excised root tips respiring in Warburg flasks the maximum effect on respiration at a given concentration was attained more rapidly with DNBP than with CIPC. The relative speed and extent of reaction of DNBP may be partially attributed to its greater solubility in water. The fact that a steady rate of respiration was not reached with CIPC within the three hour experimental period may indicate a slow but continual absorption of the chemical with an accumulative effect on oxygen consumption.

## SUMMARY

1. The effects of two important pre-emergence herbicides, an alkanolamine salt of dinitro ortho secondary butyl phenol and isopropyl N-(3-chlorophenyl)carbamate, on the germination, root elongation and root tip respiration of cotton seeds and seedlings were studied.

2. Germination and root elongation were strongly inhibited by low rates of application of DNBP in contact with germinating cotton seed. CIPC did not inhibit germination of cotton seed. However, root elongation was inhibited a constant amount throughout the range of rates used.

3. Respiration of cotton root tips excised from seedlings grown in contact with the two chemicals indicated a slight stimulatory effect for sublethal dinitro and a progressive depression of respiration with increasing rates of CIPC.

4. The effects of the dinitro compound and CIPC on the respiration of previously untreated root tips are presented. DNBP was rapidly inhibitory and the inhibition due to CIPC was more gradual. CIPC depressed $O_2$ uptake more than $CO_2$ evolution, with a resultant high RQ. The possibility that aerobic fermentation was induced was discussed.

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# Effect of Maleic Hydrazide on Soil Bacteria and other Microorganisms

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## INTRODUCTION

WITHIN the past few years the use of chemicals as herbicides has increased so rapidly that today in the United States alone millions of acres are being treated in the fight to control weeds. In addition to determining the effect of these chemicals on the plants involved (both desirable ones as well as weeds), it is most important to find out how the compounds affect the soil microflora. This assemblage of microscopic organisms plays a most vital role in the balance of nature, and, if the herbicide to be employed has as great an effect on the soil population as it does on the higher plants, its use might be impractical due to the resultant imbalance.

The best known of the herbicides, as well as the most widely used, is 2,4-D (2, 4-dichlorophenoxyacetic acid). Many workers have tested its effects on microorganisms, and the general conclusion is that at the levels used for herbicidal activity, 2,4-D has little or no adverse effect on their growth.

Among the specific organisms tested and found to be relatively unaffected by 2,4-D were the free nitrogen fixing *Azotobacter* (21) and the symbiotic nitrogen fixing *Rhizobium* (2,13,23). Kratochvil made a comparative study of the effects of several established as well as several experimental herbicides on soil microorganisms (9). He found that those tested fell into three groups: (a) those causing a significant reduction in soil microbial activity:TCA (trichloroacetic acid), PCP (pentachlorophenol), IPC (isopropyl-N-phenylcarbamate), EH2 (dichloralurea), (b) those having no influence: 2,4-D, Endothal (disodium 3,6-endoxohexahydrophthalate), 2,4,5-T (2,4,5-trichlorophenoxyacetic acid), and (c) those having a stimulatory effect on microbial activity: EHl (sodium 2,4-dichlorophenoxyethyl sulfate).

One of the more recent additions to the groups of compounds which control plant growth is the growth inhibitor maleic hydrazide (MH) (19). This compound is not a plant hormone in the conventional sense of the term, but rather seems to be a true growth inhibitor, being unable to stimulate at high dilutions (16,17,20,22). Evidence is accumulating that it acts as an anti-auxin (10,11).

Work recently published, which was done with pathological as well as normal plant material, showed that at very low concentrations, MH inhibits the growth of *Rumex* virus tumor tissue grown *in vitro* (17). During the same period work was started to test its effect on soil bacteria. It is the purpose of this paper to present data on this subject. While this material was being written, an article appeared by Daste (5) showing that MH is practically without effect

on the development of *Azotobacter* in the soil, whereas it is strongly inhibitory to *Cytophaga*. The present paper will furnish data to support the lack of effect on *Azotobacter* until high levels are reached.

## MATERIALS AND METHODS

Five widely different types of organisms were used in these tests: (1) the free-living nitrogen fixing *Azotobacter vinelandii*, (2) the crown gall organism *Agrobacterium tumefaciens*, (3) a group of microorganisms representing the various groups affecting animals and man, including Gram-positive, Gram-negative, and acid fast bacteria as well as a yeast-like fungus, (4) pure cultures of unidentified bacteria freshly isolated from the soil, including Gram-positive and Gram-negative types, and (5) nine strains representing 5 species of the symbiotic nitrogen fixing genus *Rhizobium*. Since the conditions for working with each group mentioned above are so different, it is deemed best to give the methods used with each type under the experimental results for that type.

The maleic hydrazide used in this work was in the form of the diethanolamine salt in 30 per cent solution, supplied by Naugatuck Chemical Division, U. S. Rubber Company. The levels used are based on the active ingredient.

## EXPERIMENTAL RESULTS

### 1. *Azotobacter vinelandii*

The strain of *Az. vinelandii* and minimal solution used for its maintenance were the same as used by Nickell and Burkholder in their study of the effect of antibiotics on *Azotobacter* (18). The composition of this minimal solution is as follows: $K_2HPO_4$, 0.8 gram; $KH_2PO_4$, 0.2 gram; $MgSO_4$, 0.2 gram; NaCl, 0.2 gram; $CaSO_4$, 0.1 gram; $Fe_2(SO_4)_3$, 0.01 gram; sucrose, 20.0 grams; trace elements solution (1), 0.5 ml.; and water, 1000 ml. The pH of this medium was 7.0.

Two types of experiments were conducted. In the first type MH was added in the desired concentrations to the minimal medium containing 1.5% Difco Noble agar and autoclaved. This was poured into Petri plates and let solidify. Then 1 ml. of a fresh suspension of *Azotobacter* at a dilution which would permit growth of separate colonies in the control was plated out on this medium. The effect of MH on the resultant number of colonies is shown in Table I. These counts were made after 72 hours incubation.

*Table 1.* Effect of MH on the growth of *Azotobacter vinelandii*. MH added to solid minimal medium, *Az. vinelandii* plated out on solidified medium. Number of colonies counted after 72 hours.

| MH added | No. colonies per plate |
|---|---|
| Control | 570 |
| 10 ppm | 655 |
| 100 ppm | 465 |
| 1000 ppm | 0 |

In the second type of experiment MH was added in the desired concentration to the liquid minimal medium. After sterilization of the medium, tubes were inoculated with a fresh suspension of *Azotobacter* and placed on a shaker at 21°C. for seven days. At this time, 1 ml. was removed from each test level and plated out on fresh solidified minimal medium containing *no* MH. After 72 hours, counts were made to determine the effect of MH on the growth of *Az. vinelandii* under these conditions (Table II).

*Table 2.* Effect of MH on the growth of *Azotobacter vinelandii.* MH added to liquid minimal medium, inoculated with *Az. vinelandii,* shaken at 21°C. for seven days, plated out on solidified medium without MH. Number of colonies counted after 72 hours.

| MH added | No. colonies per plate |
|---|---|
| Control | 312 |
| 1 ppm. | 346 |
| 10 ppm. | 270 |
| 100 ppm. | 180 |
| 1000 ppm. | 130 |

## 2. *Agrobacterium tumefaciens*

The strain of *A. tumefaciens* used was the A6 strain of Riker. The medium used throughout the experiments with this organism was Difco nutrient broth or agar according to the type experiment.

The same two types of experiments were conducted as with *Az. vinelandii:* (1) MH added to nutrient broth, inoculated with the crown gall bacterium, incubated at 21°C. for 60 hours on a shaker, plated out on nutrient agar, colonies counted after 48 hours incubation at 21°C.; and (2) fresh suspension of the crown gall bacterium plated out on nutrient agar containing desired levels of MH and incubated 48 hours at 21°C.

Results of the first experiment are shown in Table III. In the second type of experiment, levels of MH in the agar up to 10,000 ppm had no effect on the number of colonies of the bacteria. At 50,000 ppm there was complete inhibition of growth.

*Table 3.* Effect of MH on the growth of *Agrobacterium tumefaciens.* MH added to nutrient broth, inoculated with *A. tumefaciens,* shaken at 21°C. for 60 hours, plated out on nutrient agar without MH. Number of colonies counted after 48 hours.

| MH added | No. colonies per plate |
|---|---|
| Control | 964 |
| 1,000 ppm. | 194 |
| 10,000 ppm. | 121 |
| 50,000 ppm. | 0 |

A third type of experiment was carried out with MH and *A. tumefaciens* in which the bacteria were inoculated into cooled nutrient agar, poured into Petri plates, and let solidify. Then filter paper discs were dipped into MH solutions of various concentrations and placed in the center of the plates which were then incubated for 48

hours at 21°C. After this incubation the control plate and those containing pads with MH solutions up through 10,000 ppm had a complete film of bacterial growth. Those containing pads with MH solutions of 50,000 ppm had no zones of inhibition but rather small discrete colonies uniformly distributed throughout the plate, showing an inhibition of what would be normal growth of the original inoculum cells.

3. *Representative microorganisms*

Microorganisms, which represent a variety of genera and species, of a group commonly used for routine antibiotic testing were selected for study (6). The sensitivity of these microorganisms to MH was determined by the two-fold, serial dilution technique. Final concentration of MH ranged from 100 ppm in the first tube diluted through 0.5 ml. of medium to 0.19 ppm in the tenth tube. The inoculum consisted of 0.5 ml. of a $1x10^{-3}$ dilution of a standardized culture. Final volume in each tube was 1 ml. Incubation was at 37°C. for 18–24 hours. The medium of choice for the regular laboratory stock cultures was Difco nutrient broth. The medium of Dubos adjuncted with Tween 80 was used for the tests involving the saprophytic mycobacteria. Incubation for the saprophytic mycobacteria was at 37°C. for approximately 36 hours. The microorganisms used in this experiment are listed in Table IV. In concentrations up to 100 ppm, MH had no activity against any of these organisms.

*Table 4.* List of microorganisms against which MH was found to be inactive at the levels tested.

| | |
|---|---|
| *Aerobacter aerogenes* strain No. 1 (antibiotic resistant) | *Klebsiella pneumoniae* |
| *Aerobacter aerogenes* strain No. 2 | *Micrococcus pyogenes* var. *aureus* |
| *Aerobacter aerogenes* parent strain | *Bacillus subtilis* |
| *Escherichia coli* | *Streptococcus faecalis* |
| *Proteus* sp. | *Candida albicans* |
| *Pseudomonas aeruginosa* | *Mycobacterium ranae* |
| *Salmonella typhosa* | *Mycobacterium phlei* |
| *Salmonella paratyphi A* | *Mycobacterium smegmatis* |
| *Salmonella paratyphi* B | *Mycobacterium* sp. No. 607 |
| *Brucella bronchiseptica* | *Mycobacterium butyricum* |
| | *Mycobacterium berolinense* |

4. *Unidentified bacteria freshly isolated from the soil*

The bacteria used in this experiment were freshly isolated into pure cultures from soil samples collected in different localities and different soil types. They were not identified further than their staining by the Gram method. Both Gram-positive and Gram-negative types were represented. The only effort made to vary the isolates was based on colonial appearance, color, and other gross characters. From the many isolates, 10 which differed in these gross characteristics were used in tests with MH. The technique was the same two-fold serial dilution method described in the previous section. In no instance was there any activity shown against the soil bacteria. For this reason, a further experiment was conducted in order to determine how high a concentration of MH was necessary to prevent growth of these bacteria. In the case of isolate #14484–22 there was no effect on growth at 9 mgm/ml or 9,000 ppm. At 18,000 ppm

growth was inhibited. In the case of isolate #14489–32, there was no effect on growth at 18,000 ppm. At 36,000 ppm growth was inhibited.

5. *Strains of Rhizobium species*

The sensitivity of these bacteria was determined by the same two-fold serial dilution method described in the two previous sections. The results presented in Table V show that certain strains of the rhizobia are the most sensitive soil microorganisms tested.

*Table 5.* Effect of MH on the growth of *Rhizobium* strains. Minimum inhibitory concentration (MIC) determined by two-fold, serial dilution technique.

| Strain | MIC |
|---|---|
| *Rhizobium lupini* 814 | 280 |
| *Rhizobium leguminosarum* 317 | 570 |
| *Rhizobium lupini* 810 | 280 |
| *Rhizobium* cowpea 629v | 2300 |
| *Rhizobium japonicum* 500v | 280 |
| *Rhizobium leguminosarum* 308 | 1150 |
| *Rhizobium phaseoli* D-10-1 | 280 |
| *Rhizobium japonicum* 504v | 570 |
| *Rhizobium phaseoli* 405 | 570 |

## DISCUSSION AND SUMMARY

Because of the possibility of its use as a general herbistat and as a growth inhibitor of grasses, maleic hydazide has been tested for its effect on microorganisms, particularly soil bacteria. In general, MH was found to have little effect on the growth of these microorganisms. Even the most sensitive organisms tested, certain strains of *Rhizobium,* were unaffected until levels of 280 ppm and over were reached. All other bacteria were relatively unaffected until levels of 1000 ppm and over were reached. This was with MH added to the growth medium, thereby giving it full opportunity to act. It is not known whether, at the higher concentrations affecting bacterial growth, it is actually MH which causes the inhibition. Perhaps the diethanolamine is responsible. Chronic feeding studies in animals indicate that diethanolamine itself is toxic (25).

It is therefore logical to assume that MH is a safe compound to use in the regulation of plant growth, since the levels used effectively on plants are lower in most cases than that level which affects bacterial growth (3,4,7,8,14–16,19,24). Furthermore, in most cases, application is by spraying, so that the total amount reaching the ground is far less than that used in the spray since less spray reaches the ground. Once in the soil, the level would probably be further lowered by leaching, physical inactivation, chemical inactivation, and biological degradation as is indicated by the work of Levi and Crafts (12). The conclusion reached by these workers is that MH residues would not constitute a problem in most California soils.

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# Effect of CMU on Nutgrass *(Cyperus rotundus* L.)

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Since CMU (3-(para-chlorophenyl)-1,1-dimethylurea) has been shown to be a potent herbicide which persists in the soil for long periods of time (1, 2, 3) it was tested in the following experiments to control nutgrass *(Cyperus rotundus* L.).

## Experimental

A flat area with a heavy stand of nutgrass was divided into two randomized blocks that received four treatments each. One block was plowed to a depth of 6 inches and the other block was left undisturbed. CMU was applied in the form of 80 percent wettable powder with a knapsack sprayer at the rate of 0, 20, 40, and 80 pounds per acre to plots in each block.

Samples of soil 1-foot-square at 3-inch intervals to a depth of 9 inches were taken from the various plots before the treatments were started and again in November, 5½ months after the herbicide was applied.

Within a month after treatment the nutgrass in all CMU plots appeared chlorotic and began to die off. By the end of the second month the nutgrass had disappeared in all treated plots except those unplowed and sprayed at the 20-pound rate. In these latter plots the nutgrass was only partially killed. Heavy rain fell during the next 3 months and the nutgrass in all unplowed plots regardless of rate of CMU gradually recovered until the land was completely covered with the weed. Nutgrass in the plots plowed and sprayed with 20 pounds of CMU per acre also recovered completely while that in plots sprayed with 40 pounds of CMU recovered partially and nutgrass in plots treated with 80 pounds of CMU recovered only slightly.

Although there was a reduction of 80 to 90 percent in number of live tubers in the plots plowed and treated with 80-pound CMU per acre, enough live tubers remained to reinfest the area. This experiment indicates that a single application of CMU at a relatively high rate reduced the infestation of nutgrass but did not eradicate it.

*Effect of CMU on nutgrass tubers at different depths in the soil.* – Studies on the movement of CMU in the soil have shown that the herbicide does not move beyond the first two inches even when an inch rainfall equivalent followed its application (3).

In view of that fact, the following experiment was conducted to study the effect of depth of planting of nutgrass and treatment with CMU, under greenhouse conditions where environmental factors could be better controlled.

Mature, healthy tubers were collected from a plowed field and divided at random into lots of 20 tubers each. Five-gallon earthenware jars (coffee urns) were filled with a uniform mixture of screened

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*Table 1.* Effect of soil surface treatment with CMU on growth and propagation of nutgrass tubers planted 1″ to 15″ in depth.[1]

| Planting depth of 20 tubers 1/11/52 | Shoots per jar | | Tubers per jar | | | | Total fresh weight of tubers per jar | | Average fresh weight per tuber | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Alive | | Dead | | | | | |
| | Check | Treated | Check | Treated | Check | Treated | Check | Treated | Check | Treated |
| | No. | No. | No. | No. | No. | No. | Gms. | Gms. | Mgs. | Mgs. |
| 1 in... | 53 | 0 | 184 | 0 | 0 | 11 | 54.1 | 0 | 294 | 0 |
| 2 in... | 48 | 0 | 234 | 0 | 0 | 15 | 64.5 | 0 | 275 | 0 |
| 4 in... | 45 | 11 | 223 | 31 | 0 | 11 | 52.2 | 2.4 | 234 | 77 |
| 6 in... | 62 | 3 | 215 | 22 | 0 | 13 | 50.8 | 3.9 | 236 | 177 |
| 8 in... | 47 | 5 | 235 | 15 | 1 | 17 | 55.0 | 2.8 | 234 | 186 |
| 10 in... | 33 | 9 | 141 | 15 | 0 | 18 | 23.5 | 3.2 | 166 | 213 |
| 12 in... | 45 | 6 | 130 | 8 | 4 | 18 | 22.1 | 2.6 | 170 | 325 |
| 15 in... | 30 | 16 | 155 | 59 | 1 | 10 | 31.4 | 9.0 | 202 | 152 |

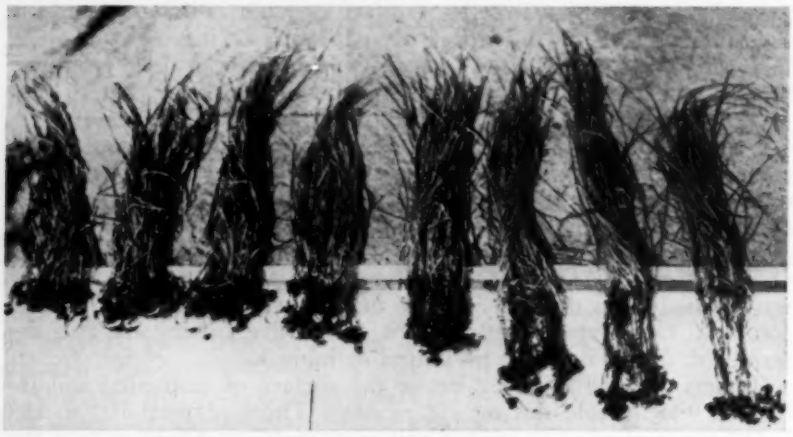
[1]Data obtained June 27, 1952, 5½ months after planting and treatment.

soil free of nutgrass tubers. Duplicate sets of jars were planted with 20 tubers each at depths of 1, 2, 4, 6, 8, 10, 12, and 15 inches below the surface. After planting, the required depth of soil was placed on top of the tubers and firmed gently without packing. All jars were then watered to field capacity or until excess water drained from the holes at the bottom of the jars. One member of each pair of jars was treated with CMU at the rate of 40 pounds per acre. The required amount of CMU was dispersed in water and sprayed on to the surface of the soil. After the treatments were applied and at intervals of 1 week or as needed, all the jars were watered with the equivalent of one-fourth inch of rain with a sprinkling can. Emergence counts and other data were taken at 1, 2, and 5½ months after treatment. At the time the final data were obtained, the soil was washed from the jars and the number of live and dead tubers counted. The average fresh weight of the live tubers was also determined. These data are presented in table 1.

Tubers planted 1″ to 8″ below the surface of untreated soil increased 9 to 11 fold during 5½ months. Those planted 10″ to 15″ below the surface of untreated soil increased 7 to 8 fold during the same period. All tubers appeared healthy regardless of depth of planting, but the tubers developed from those planted 1″ to 8″ below the surface were larger and heavier than those developed from tubers planted 10″ to 15″ deep.

The tubers planted 1″ and 2″ below the surface of treated soil were completely eradicated. Those planted at a 4″ depth increased in numbers, but they were very small and shriveled as evidenced by the average weight per tuber (77 mg.), compared to that of the corresponding check tubers, (234 mg.). Tubers planted 6″ to 12″ below treated soil showed little or no increase in numbers during the 5½ months, but instead there was considerable decrease at most planting depths. However, as the planting depth increased the size and weight of the surviving tubers increased. Tubers planted 15″ below treated soil increased three-fold in number, and these appeared healthy though somewhat smaller than the corresponding check tubers.

These data show that CMU can eradicate nutgrass tubers within 4″ of the soil surface. They also show that CMU has a strong inhibitory effect on tubers located as deep as 15″ below the surface. However, the deeper the tubers are located the better chance they have of surviving the effects of the CMU. Eventually the toxic effect of the CMU is dissipated and the deep tubers can sprout and reinfest an area. Therefore, an effective program for eradication of nutgrass with CMU should include occasional deep plowings to bring the tubers to the surface and in contact with the CMU. Figure 1 shows nutgrass plants that developed from tubers planted in the soil at depths of 1, 2, 4, 6, 8, 10, 12, and 15 inches. The plants were harvested four months after planting. Note that the above-ground height of shoots is about the same regardless of the depth at which the tubers were planted. No plants developed in the pots treated with CMU and planted with tubers at 1 and 2 inch depths and only a few plants developed from the other treated pots.



Depth of planting (inches)

FIG. 1. — Shows nutgrass plants that developed from tubers planted in untreated soil at depths of 1, 2, 4, 6, 8, 10, 12, and 15 inches. The plants were harvested 4 months after planting. Note that the above-ground height of the shoots is about the same regardless of the depth at which the tubers were planted.

*Effect of CMU and alternate plowing on nutgrass control.* — The foregoing field experiment showed that high rates of CMU (80 pounds per acre) greatly reduced the population of nutgrass tubers in the soil but did not completely eradicate them. Field and greenhouse studies indicated that the reason for the incomplete eradication was due to the fact that CMU did not leach beyond the first two inches of soil (3) and the deep tubers were protected against the toxic action of the herbicide near the surface. Thus, the tubers that did not come in contact with CMU remained viable until the toxicity in the surface two inches of soil had dissipated. In view

of this fact the following experiment was carried out in which relatively low rates of CMU were combined with alternate plowing to bring the tubers in contact with the herbicide. Plots treated with 2,4-D and TCA (sodium trichloroacetate) were also included in the experiment for comparison.

A flat area of toa silty clay soil infested with nutgrass was plowed, disked and divided into two randomized blocks that received the treatments listed in table 2. The herbicides were applied in water with knapsack sprayers. Six weeks after the initial application all the plots except the undisturbed control plots were plowed again and retreated at the indicated rates. After 14 weeks one half of each treated plot was plowed and sprayed again. Counts of live tubers to a depth of 12 inches were made in December.

The data (table 2) show that six months after the initial treatment all plots in which CMU was applied either alone or in combination with 2,4-D and TCA had only a few live tubers remaining. On the other hand, check plots and those treated only with TCA had over 600 tubers per cubic foot and plots treated only with 2,4-D had 250 tubers per cubic foot at the end of six months.

*Table 2.* Counts of live nutgrass tubers per cubic foot of soil, 6 months after treatment with CMU, TCA, 2,4-D alone and combined.

| Treatments | Two plowings—6 weeks apart each followed by application at indicated rate | Three plowings—6 and 8 weeks apart each followed by application at indicated rate |
|---|---|---|
| | Tuber counts per cu. ft. | Tuber counts per cu. ft. |
| | No. | No. |
| Control (plowed) | 501 | —† |
| Control (not plowed)* | 621 | —† |
| 40 lb./a CMU | 7 | 4 |
| 20 lb./a CMU | 23 | 5 |
| 20 lb. CMU<br>20 lb. TCA | 5 | 3 |
| 20 lb. CMU<br>20 lb. TCA<br>5 lb. 2,4-D | 25 | 5 |
| 20 lb. CMU<br>5 lb. 2,4-D | 3 | 2 |
| 40 lb. TCA | 735 | 615 |
| 5 lb. 2,4-D | 131 | 250 |

*Average number tubers per cu. ft. at start of experiment *170*.
†No data taken.

This experiment shows that nutgrass tubers were reduced as effectively in plots plowed and sprayed twice with 20 pounds CMU as in plots plowed and treated 2 or 3 times with 40 pounds CMU. The applications of relatively low rates of CMU combined with alternate plowings was more effective in killing the tubers than was application of high rates of CMU alone. Figure 2 shows the good control of nutgrass achieved with two applications of CMU each at 20 pounds per acre followed by two plowings 6 weeks apart. The photograph was taken 4 months after the last plowing. Examination of the soil to a depth of 12″ showed only an occasional live tuber.

FIG. 2. — Control of nutgrass was achieved with two applications of CMU each at 20 pounds per acre followed by two plowings 6 weeks apart. The photograph was taken 4 months after the last plowing. Examination of the soil to a depth of 12″ showed only an occasional live tuber. Note the nutgrass in the foreground on a plowed but untreated plot.

## SUMMARY

Preliminary field experiments designed to test CMU for controlling nutgrass showed that there was 80 to 90 percent reduction in numbers of live tubers in plots that had been plowed and then treated with the herbicide at the rate of 80 pounds per acre.

A greenhouse experiment in which nutgrass tubers were planted 1, 2, 4, 6, 8, 10, 12, and 15 inches below the surface of soil treated with CMU at 40 pounds per acre and in untreated soil showed that tubers planted 1″ and 2″ below the surface of treated soil were completely eradicated and those planted at a 4″ depth were shriveled and apparently severely damaged. Tubers planted 6 to 12 inches below treated soil decreased in numbers during 5½ months after treatment but those that remained appeared healthy. Tubers planted at a depth of 15″ increased three-fold in number though they were smaller than the tubers planted at the same depth in untreated soil. Tubers planted in untreated soil increased many fold in number at all depths of planting. This and other studies which showed that CMU did NOT move beyond the first two inches of soil indicated the reason for the incomplete tuber eradication even when 80 pounds per acre of CMU was used.

Subsequent field experiments in which low rates of CMU were

combined with alternate plowing to bring the tubers in contact with the herbicide showed that nutgrass tubers were practically eradicated in plots that had been plowed and sprayed twice – 6 weeks apart with CMU at 20 pounds per acre.

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# Failure of Light Activated Riboflavin to Counteract the Growth Inhibition of Two Benzoic Acid Derivatives[1]

ROBERT H. HAMILTON and RICHARD J. ALDRICH[2]

IT HAS been reported by Carroll (1, 2) that light activated riboflavin will counteract 2,4-D inhibition, presumably due to an inactivation or destruction of the 2,4-D molecule. Hansen and Buchholtz (7) found that light activated riboflavin would inactivate the 2,4-D molecule in vitro and it appeared that 2,4-dichlorophenol was one of the decomposition products. Galston (3, 4, 5) has shown that riboflavin may act as a photo-receptor and a hydrogen carrier in the oxidation of indol-3-acetic acid, the reduced riboflavin being reoxidized by the air. It would appear, therefore, that riboflavin may play an important role in the breakdown of growth substances in the plant.

Plants treated with benzoic acid derivatives show prolonged effects as compared with 2,4-D. Minarik, et al (8) found that while 2,4-D treatment resulted in 3 or 4 malformed leaves on young bean plants, the benzoic acid derivatives resulted in a much greater number of distorted leaves, indicating persistence of the latter materials in the plant. The authors also have noted in field tests that chlorinated benzoic acid derivatives appear to be very persistent in sensitive plants. Tests were undertaken to determine whether light activated riboflavin would inactivate certain chlorinated benzoic acid derivatives.

Sixteen ml of a triethanolamine salt of 2,4-D, 2,3,5-triiodobenzoic acid (TIBA), and the ethyl ester of trichlorobenzoic acid (ethyl TCB) were placed in 6 inch pyrex test tubes alone at 50 ppm and in mixture with 500 ppm of riboflavin. The test tubes were illuminated for 48 hours with 30-watt fluorescent tubes placed about 2 feet above the test tubes. After illumination the solutions were transferred to 6 inch petri dishes containing filter paper. Twenty-five cucumber seeds were planted in each dish. The root and shoot length of the seedlings was recorded following germination for 96 hours at 29°C. This test (9) has been used successfully in assaying the benzoic acid derivatives at concentrations above 1 ppm.

Table 1 shows that riboflavin alone at 500 ppm stimulated cucumber roots to some extent. Riboflavin inactivated 2,4-D at 50 ppm which agrees with reports of investigators cited previously. However, it is clear that riboflavin did not inactivate the two benzoic acid derivatives; in fact there was a significant decrease greater than twice the sum of the standard errors where riboflavin was added in each case.

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If riboflavin does play a major role in the inactivation of growth substances by plants, the apparent long persistence of the benzoic derivatives in plants might be due to their failure to form an oxidation product with riboflavin or a flavoprotein. It would be logical perhaps to associate the riboflavin inactivating system with physiological selectivity of the growth regulating herbicides. That is, perhaps a monocot resistant to 2,4-D has a better inactivating mechanism than the broad leaved plant. If this were the case the benzoic derivatives should be relatively nonselective. However, these derivatives have been found to be somewhat more selective than 2,4-D (6).

*Table 1.* The root and shoot length of cucumber seedlings germinated in 2,4-D, TIBA, and ethyl TCB solutions which were illuminated 48 hours with and without riboflavin prior to germination.

| Treatment | Number of seedlings | Root length and standard error | Shoot length and standard error |
|---|---|---|---|
| | | mm | mm |
| Control | 102 | 71.83 ± 1.90 | 44.49 ± 0.90 |
| Riboflavin 500 ppm | 101 | 92.70 ± 2.31 | 44.63 ± 0.84 |
| 2,4-D | 88 | 5.83 ± 0.14 | 5.05 ± 0.29 |
| 2,4-D + riboflavin | 83 | 33.39 ± 1.33 | 40.06 ± 1.25 |
| Ethyl TCB | 97 | 18.94 ± 0.90 | 25.98 ± 1.37 |
| Ethyl TCB + riboflavin | 92 | 13.07 ± 0.72 | 16.26 ± 0.71 |
| TIBA | 46 | 7.06 ± 0.66 | 0 |
| TIBA + riboflavin | 41 | 5.02 ± 0.34 | 0 |

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## Coming Events

The first National Weed Conference will be held in Kansas City, Missouri, December 8–10, 1953. Headquarters will be in the Muehlbach Hotel.

The Northeastern Weed Control Conference will be held in New York City, January 6–8, 1954. Headquarters will be in the Hotel New Yorker.

The Southern Weed Conference meets at the Peabody Hotel in Memphis, Tennessee, January 11–13, 1954.

The Western Canadian Weed Control Conference is to be held at the MacDonald Hotel, Edmonton, Alberta, November 26–27, 1953.

The First National Weed Control Conference (Great Britain) will be held November 3–5, 1953, at the St. Georges and Norfolk Hotels, Cliftonville, Margate.

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This bibliography of current literature on weeds and their control is issued as a service to those engaged in weed research in the United States and other countries. In issuing this bibliography the Division of Weed Investigations does not assume responsibility for the subject matter cited.

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GENEVA, N. Y.